# REPORT

## DEVELOPMENT AND VALIDATION OF AN ANALYTICAL METHOD FOR

[redacted]

**NOTOX Project 338805**
**NOTOX Substance 111834/B**

## STATEMENT OF GLP COMPLIANCE

NOTOX B.V., 's-Hertogenbosch, The Netherlands

The study described in this report has been correctly reported and was conducted in compliance with the most recent edition of:

The OECD Principles of Good Laboratory Practice

which are essentially in conformity with:

The United States Food and Drug Administration. Title 21 Code of Federal Regulations Part 58.

The United States Environmental Protection Agency (FIFRA). Title 40 Code of Federal Regulations Part 160.

The United States Environmental Protection Agency (TSCA). Title 40 Code of Federal Regulations Part 792.

## CONFIDENTIALITY STATEMENT

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Study Director

[redacted]

Date: 18 JUL 2002

Management

[redacted]
Section Head
Analytical & Physical Chemistry

Date: July 18, 2002

QUALITY ASSURANCE STATEMENT

NOTOX B.V., 's-Hertogenbosch, The Netherlands

This report was audited by the NOTOX Quality Assurance Unit to ensure that the methods and results accurately reflect the raw data.

The dates of Quality Assurance inspections and audits are given below.
During the on-site inspections procedures applicable to this type of study were inspected.

| DATES OF QAU INSPECTIONS/AUDITS | REPORTING DATES |
|---|---|
| **on-site inspection (s)** | |
| 18 February – 01 March 2002 (Process, physical chemistry) | 04 March 2002 |
| 13 May – 31 May 2002 (Process, physical chemistry) | 04 June 2002 |
| **protocol inspection (s)** | |
| 06 November 2001 (Study) | 06 November 2001 |
| **report audit (s)** | |
| 13 June -18 June 2002 (Study) | 18 June 2002 |

Head of Quality Assurance
[redacted]

Date: 19-7-02

SUMMARY

Analytical method

A high performance liquid chromatographic (HPLC) method for quantitative analysis of the test substance [redacted]) was developed. A Zorbax RX-C18 column was used and a spectrophotometric detector set to read the absorbance at 260 nm for MIPK and 220 nm for all other components. During each run, the initial mobile phase of 46/54 (v/v) acetonitrile/Milli-Q water was changed gradually to 100 % acetonitrile by a gradient program.

Standard solutions of the test substance were prepared in acetonitrile at exactly known concentrations between 10950 mg/l and 23750 mg/l. In order to obtain suitable concentrations for the validation tests, these solutions were further diluted with 50/50 (v/v) acetonitrile/Milli-Q water.

Several small test substance peaks and one large test substance peak were observed in chromatograms of test substance solutions. The area of the small peaks was used as test substance response in calculations during the validation tests. The large peak corresponds to Dimethylphthalate which was used for preparation of this formulation. This peak was not used during method validation and not reported here since it cannot be analysed in the same run together with the smaller peaks due to the large difference in sensitivity. Peak identification was based on information supplied by the sponsor.

The HPLC method was validated for repeatability of injections, stability of the chromatographic system, stability of standard solutions, linearity and limit of detection.

Repeatability of injections based on [redacted]

The coefficient of variation of the response was determined to be 3.0 %, 1.6 % and 0.2 % for 10 replicates of a single 106 mg/l solution, a single 212 mg/l solution and a single 10575 mg/l solution, respectively. Therefore it was concluded that the chromatographic and integration conditions are acceptable for further analysis in the concentration range 212 - 10575 mg/l. Due to a relatively large coefficient of variation, it was necessary to pay extra attention to integration conditions at concentrations below 212 mg/l.

Repeatability of injections based on [redacted]

The coefficient of variation of the response was determined to be 0.2 %, 0.5 % and 0.2 % for 10 replicates of a single 106 mg/l solution, a single 212 mg/l solution and a single 10575 mg/l solution, respectively. Therefore it was concluded that the chromatographic and integration conditions are acceptable for further analysis in the concentration range 106 - 10575 mg/l.

Repeatability of injections based on [redacted]

The coefficient of variation of the response was determined to be 6.5 %, 3.9 % and 0.3 % for 10 replicates of a single 106 mg/l solution, a single 212 mg/l solution and a single 10575 mg/l solution, respectively. Due to a relatively large coefficient of variation, it was necessary to pay extra attention to integration conditions below 10575 mg/l.

Repeatability of injections based on [redacted] peak 1

The coefficient of variation of the response was determined to be 1.2 %, 0.7 % and 0.1 % for 10 replicates of a single 106 mg/l solution, a single 212 mg/l solution and a single 10575 mg/l solution, respectively. Therefore it was concluded that the chromatographic and integration conditions are acceptable for further analysis in the concentration range 106 - 10575 mg/l.

Repeatability of injections based on [redacted] peak 2

The coefficient of variation of the response was determined to be 0.8 %, 0.5 % and 0.1 % for 10 replicates of a single 106 mg/l solution, a single 212 mg/l solution and a single 10575 mg/l solution, respectively. Therefore it was concluded that the chromatographic and integration conditions are acceptable for further analysis in the concentration range 106 - 10575 mg/l.

Stability of the chromatographic system based on all components

The chromatographic system was stable over at least a 22.8-hour time interval at a test substance concentration of 10575 mg/l.

Stability of standard solutions based on all components

Standard solutions (11350 mg/l and 21460 mg/l) of the test substance in acetonitrile were stable for 24 days when stored at room temperature in the dark.

Linearity based on [redacted]

A linear relationship between response and test substance concentration was found over a concentration range of 21.2 – 10575 mg/l if a (1/concentration) weighting factor was applied (n = 9, r = 0.9998). Unweighted regression resulted in a deviation of more than 10% of the calibration points from the calculated line at concentrations below 212 mg/l.

Linearity based on [redacted]4

A linear relationship between response and test substance concentration was found over a concentration range of 10.6 – 10575 mg/l if a (1/concentration) weighting factor was applied (n = 10, r = 0.9998). Unweighted regression resulted in a deviation of more than 10% of the calibration points from the calculated line at concentrations below 212 mg/l.

Linearity based on [redacted]

A linear relationship between response and test substance concentration was found over a concentration range of 52.9 – 10575 mg/l if a (1/concentration) weighting factor was applied (n = 8, r = 0.9997). Unweighted regression resulted in a deviation of more than 10% of the calibration points from the calculated line at concentrations below 212 mg/l.

Linearity based on [redacted] peak 1

A linear relationship between response and test substance concentration was found over a concentration range of 10.6 – 10575 mg/l if a (1/concentration) weighting factor was applied (n = 10, r = 0.99994). Unweighted regression resulted in a deviation of more than 10% of the calibration points from the calculated line at concentrations below 212 mg/l.

Linearity based on [redacted] peak 2

A linear relationship between response and test substance concentration was found over a concentration range of 10.6 – 10575 mg/l if a (1/concentration) weighting factor was applied (n = 10, r = 0.99993). Unweighted regression resulted in a deviation of more than 10% of the calibration points from the calculated line at concentrations below 212 mg/l.

Limit of detection based on [redacted] e

The limit of detection for the test substance was determined to be 0.8 mg/l at an injection volume of 100 µl.

Limit of detection based on [redacted]

The limit of detection for the test substance was determined to be 2 mg/l at an injection volume of 100 µl.

Limit of detection based on [redacted]

The limit of detection for the test substance was determined to be 46 mg/l at an injection volume of 100 µl.

Limit of detection based on [redacted] peak 1

The limit of detection for the test substance was determined to be 11 mg/l at an injection volume of 100 µl.

Limit of detection based on [redacted] peak 2

The limit of detection for the test substance was determined to be 10 mg/l at an injection volume of 100 µl.

## PREFACE

| | |
|---|---|
| Sponsor | [redacted] |
| Study Monitor | D[redacted]<br>SHERA, Regulatory Affairs |
| Testing Facility | NOTOX B.V.<br>Hambakenwetering 7<br>5231 DD 's-Hertogenbosch<br>The Netherlands |
| Study Director | [redacted] |
| Study plan | Start: 04 December 2001<br>Completed: 17 May 2002 |

## TEST SUBSTANCE

| | |
|---|---|
| Identification<br>Chemical name<br>CAS RN | [redacted] |
| Description | Clear colourless liquid |
| Batch | 1510-14 |
| Purity | See Certificate of Analysis |
| Test substance storage | In refrigerator in the dark |
| Stability under storage conditions | Stable |
| Expiry date | 01 January 2003 |

The sponsor is responsible for all test substance data unless determined by NOTOX.

Note: **Don't heat up the test substance above 50°C**

## PURPOSE AND PRINCIPLE

The purpose of the study was to implement and validate a method for the quantitative analysis of [redacted]. A high performance liquid chromatographic (HPLC) method was developed. The method was validated for repeatability of injections, stability of the chromatographic system, stability of standard solutions, linearity and limit of detection.

## ARCHIVING

NOTOX B.V. will archive the following data for at least 10 years:
protocol, report, test substance reference sample and raw data.

## REAGENTS

| | |
|---|---|
| Milli-Q water | Tap water purified by reversed osmosis and subsequently passed over activated carbon and ion-exchange cartridges; Millipore, Bedford, MA, USA |
| Acetonitrile | HPLC-grade, Labscan, Dublin, Ireland |

## ANALYTICAL METHOD

A high performance liquid chromatographic (HPLC) method for quantitative analysis of the test substance was implemented by NOTOX B.V. The conditions are described below:

| | | | |
|---|---|---|---|
| Column | Zorbax RX-C18, 250 x 4.6 mm; $d_p$=5 µm (Chrompack, Middelburg, the Netherlands) | | |
| Mobile phase A | Acetonitrile | | |
| Mobile phase B | Milli-Q water | | |
| Gradient program | Time (min.) | %A | %B |
| | 0 | 46 | 54 |
| | 5 | 46 | 54 |
| | 10 | 100 | 0 |
| | 13 | 100 | 0 |
| | 14 | 46 | 54 |
| | 19 | 46 | 54 |
| Flow | 2 ml/min | | |
| Detection wavelength | 220 nm and 260 nm | | |
| Injection volume | 100 µl | | |

## PREPARATION OF SOLUTIONS

Standard solutions of the test substance were prepared in acetonitrile at exactly known concentrations between 10950 mg/l and 23750 mg/l. Suitable concentrations for the validation tests were obtained by dilution of these solutions with 50/50 (v/v) acetonitrile/Milli-Q water.

VALIDATION OF THE ANALYTICAL METHOD

The HPLC method was validated for repeatability of injections, stability of the chromatographic system, stability of standard solutions, linearity and limit of detection.

Repeatability of injections

Each of three solutions (106 mg/l, 212 mg/l and 10575 mg/l) was injected in tenfold into the HPLC system and the responses were recorded. The coefficient of variation of the response was calculated for each concentration.

Stability of the chromatographic system

A 10575 mg/l solution was injected (in duplicate) three times in a 22.8-hour time interval. The maximum deviation of the response was calculated.

Stability of standard solutions

Two standard solutions (11350 mg/l and 21460 mg/l) in acetonitrile were stored at room temperature in the dark and measured 24 days after preparation together with two freshly prepared standard solutions (10950 mg/l and 23750 mg/l). Prior to measurement, each of the standard solutions was diluted to an exactly known concentration of approximately 1100 mg/l. Each solution was injected in duplicate. The maximum deviation of the response factor was calculated.

Linearity

From one standard solution (21150 mg/l), ten dilutions were prepared. This resulted in a concentration range of 10.6-10575 mg/l. Each of the solutions was injected in duplicate. Responses were plotted against concentrations. A linear regression program was used to calculate the regression line from the responses and concentrations.

Limit of detection

A 10.6 mg/l and a 52.9 mg/l solution were injected in duplicate. In each chromatogram, the height (expressed in µAU) of the test substance peak was measured as well as the noise level of the system (µAU). The limit of detection was calculated from the mean peak height and the mean noise level.

DATA HANDLING

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| | |
|---|---|
| Response | Peak area of the test substance [units] |
| Response factor | Response/concentration [units∗l∗$10^3$/g] |
| Mean | $\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i$<br><br>where:<br>$x_i$ : measured value<br>$n$ : number of measurements |
| Standard deviation | $s_{n-1} = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{(n-1)}}$ |
| Coefficient of variation | (standard deviation/mean) ∗ 100% |
| Maximum deviation | [(highest - lowest)/mean] ∗ 100%<br>where 'mean' is the mean value of the highest and the lowest value. |
| Linearity | A linear regression program was used to calculate the regression line from the responses and concentrations. Linear regression analysis was performed using the least squares method. A (1/concentration) weighting factor was used.<br><br>Regression line: Y = a X +b<br>where:<br>Y : response<br>X : concentration<br>a : slope<br>b : intercept |
| Correlation coefficient | During regression analysis, the correlation coefficient (r) was calculated. |
| Limit of detection | The limit of detection is defined as the lowest concentration of test substance that can be distinguished from instrumental noise using the analytical method described.<br><br>Limit of detection= ((3 ∗ noise level)/ signal) ∗ conc.<br><br>where:<br>noise level (N) : height of the noise [µAU]<br>signal (S) : height of the test substance peak [µAU]<br>conc. : concentration of test substance [mg/l] |

RESULTS

A high performance liquid chromatographic (HPLC) method was developed for the quantitative analysis of [redacted]. The HPLC method was validated for repeatability of injections, stability of the chromatographic system, stability of standard solutions, linearity and limit of detection.

Several small test substance peaks and one large test substance peak were observed in chromatograms of test substance solutions. The area of all peaks was used as test substance response in calculations during the validation tests. Peak identification was based on information supplied by the sponsor. The large peak at t = 3.11 minutes corresponds to Dimethylphthalate which was used for preparation of this formulation. This peak was not used during method validation and not reported here since it cannot be analysed in the same run together with the smaller peaks due to the large difference in sensitivity.

The calculations for the validation tests were performed using not-rounded concentrations and responses. Therefore, some differences might be observed when calculating the statistical parameters using the values as mentioned in the tables.

Repeatability of injections

The results are summarised in Tables 1 - 5.

Table 1: Repeatability of injections based on Hydrogen peroxide.

| Concentration [mg/l] | Response[1] [units] | Standard deviation [units] | Coefficient of variation [%] |
|---|---|---|---|
| 106 | 29780 | 893 | 3.0 |
| 212 | 45851 | 747 | 1.6 |
| 10575 | 1752466 | 3540 | 0.2 |

[1] Mean of 10 replicates of a single solution.

From these results, it was concluded that the chromatographic and integration conditions are acceptable for further analysis in the concentration range 106-10575 mg/l. Due to a relatively large coefficient of variation, it was necessary to pay extra attention to integration conditions at concentrations below 212 mg/l.

Table 2: Repeatability of injections based on [redacted]

| Concentration [mg/l] | Response[1] [units] | Standard deviation [units] | Coefficient of variation [%] |
|---|---|---|---|
| 106 | 45640 | 111 | 0.2 |
| 212 | 89367 | 459 | 0.5 |
| 10575 | 4345925 | 7132 | 0.2 |

[1] Mean of 10 replicates of a single solution.

From these results, it was concluded that the chromatographic and integration conditions are acceptable for further analysis in the concentration range 106-10575 mg/l.

Table 3: Repeatability of injections based on [redacted].

| Concentration [mg/l] | Response[1] [units] | Standard deviation [units] | Coefficient of variation [%] |
|---|---|---|---|
| 106 | 923 | 60 | 6.5 |
| 212 | 1940 | 77 | 3.9 |
| 10575 | 88680 | 309 | 0.3 |

[1] Mean of 10 replicates of a single solution.

Due to a relatively large coefficient of variation, it is necessary to pay extra attention to integration conditions below 10575 mg/l.

From these results, it was concluded that the chromatographic and integration conditions are acceptable for further analysis in the concentration range 106-10575 mg/l.

Table 4: Repeatability of injections based on [redacted] peak 1.

| Concentration [mg/l] | Response[1] [units] | Standard deviation [units] | Coefficient of variation [%] |
|---|---|---|---|
| 106 | 10636 | 127 | 1.2 |
| 212 | 20600 | 153 | 0.7 |
| 10575 | 1015428 | 1446 | 0.1 |

[1] Mean of 10 replicates of a single solution.

From these results, it was concluded that the chromatographic and integration conditions are acceptable for further analysis in the concentration range 106-10575 mg/l.

Table 4: Repeatability of injections based on [redacted] peak 2.

| Concentration [mg/l] | Response[1] [units] | Standard deviation [units] | Coefficient of variation [%] |
|---|---|---|---|
| 106 | 10077 | 79 | 0.8 |
| 212 | 19738 | 94 | 0.5 |
| 10575 | 984196 | 1355 | 0.1 |

[1] Mean of 10 replicates of a single solution.

From these results, it was concluded that the chromatographic and integration conditions are acceptable for further analysis in the concentration range 106-10575 mg/l.

Stability of the chromatographic system

The results are summarised in Tables 6 - 10.

Table 6: Stability of the chromatographic system based on [redacted]

| Elapsed time [hours] | Response[1] [units] | Maximum deviation [%] |
|---|---|---|
| 0.0 | 1800553 | |
| 0.4 | 1790596 | |
| 11.6 | 1746782 | |
| 11.9 | 1748878 | 3.0 |
| 22.5 | 1753119 | |
| 22.8 | 1754237 | |

[1] For a 10575 mg/l solution.

Table 7: Stability of the chromatographic system based on [redacted]

| Elapsed time [hours] | Response[1] [units] | Maximum deviation [%] |
|---|---|---|
| 0.0 | 4475698 | |
| 0.4 | 4462955 | |
| 11.6 | 4331736 | |
| 11.9 | 4339414 | 3.3 |
| 22.5 | 4363974 | |
| 22.8 | 4364589 | |

[1] For a 10575 mg/l solution.

Table 8: Stability of the chromatographic system based on [redacted]

| Elapsed time [hours] | Response[1] [units] | Maximum deviation [%] |
|---|---|---|
| 0.0 | 90618 | |
| 0.4 | 90126 | |
| 11.6 | 88974 | |
| 11.9 | 88360 | 3.6 |
| 22.5 | 87419 | |
| 22.8 | 87422 | |

[1] For a 10575 mg/l solution.

Table 9: Stability of the chromatographic system based on [redacted] peak 1.

| Elapsed time [hours] | Response[1] [units] | Maximum deviation [%] |
|---|---|---|
| 0.0 | 1048761 | |
| 0.4 | 1050283 | |
| 11.6 | 1012958 | |
| 11.9 | 1015333 | 3.6 |
| 22.5 | 1021622 | |
| 22.8 | 1022251 | |

[1] For a 10575 mg/l solution.

Table 10: Stability of the chromatographic system based on [redacted] peak 2.

| Elapsed time [hours] | Response[1] [units] | Maximum deviation [%] |
|---|---|---|
| 0.0 | 1014356 | |
| 0.4 | 1015497 | |
| 11.6 | 982026 | |
| 11.9 | 984518 | 3.4 |
| 22.5 | 989923 | |
| 22.8 | 990744 | |

[1] For a 10575 mg/l solution.

From these results, it was concluded that the chromatographic system was stable over at least a 22.8-hour time interval at the concentration tested (10575 mg/l) for all measured components.

Stability of standard solutions

The results are summarised in Tables 11 - 15.

Table 11: Stability of standard solutions in acetonitrile based on [redacted]

| Preparation date | Measurement date | Concentration[1] [mg/l] | Response factor [units*l*$10^3$/g] | Maximum deviation [%] |
|---|---|---|---|---|
| 23-4-02 | 17-5-02 | 11350 | 167.67/167.27 | |
| 23-4-02 | 17-5-02 | 21460 | 178.07/177.80 | |
| 29-4-02 | 17-5-02 | 12500 | 178.48/176.52 | |
| 29-4-02 | 17-5-02 | 21150[2] | 159.00/184.06 | 6.4 |
| 16-5-02 | 17-5-02 | 10950 | 167.94/168.08 | |
| 16-5-02 | 17-5-02 | 23750 | 177.75/177.95 | |

[1] Concentration of the undiluted standard solution.

[2] Due to a large variation in the duplicate measurements, this solution was not used in the calculation.

From these results, it was concluded that standard solutions of the test substance (11350 mg/l and 21460 mg/l) in acetonitrile are stable for at least 24 days when stored at room temperature in the dark based on Hydrogen peroxide.

Table 12: Stability of standard solutions in acetonitrile based on [redacted].

| Preparation date | Measurement date | Concentration[1] [mg/l] | Response factor [units*l*$10^3$/g] | Maximum deviation [%] |
|---|---|---|---|---|
| 23-4-02 | 17-5-02 | 11350 | 452.46/453.36 | |
| 23-4-02 | 17-5-02 | 21460 | 442.22/442.77 | |
| 29-4-02 | 17-5-02 | 12500 | 441.27/441.96 | |
| 29-4-02 | 17-5-02 | 21150 | 452.51/453.30 | 2.8 |
| 16-5-02 | 17-5-02 | 10950 | 440.73/440.88 | |
| 16-5-02 | 17-5-02 | 23750 | 446.86/447.16 | |

[1] Concentration of the undiluted standard solution.

From these results, it was concluded that standard solutions of the test substance (11350 mg/l and 21460 mg/l) in acetonitrile are stable for at least 24 days when stored at room temperature in the dark for MIPKP-T4.

Table 13: Stability of standard solutions in acetonitrile based on [redacted].

| Preparation date | Measurement date | Concentration[1] [mg/l] | Response factor [units*l*10$^3$/g] | Maximum deviation [%] |
|---|---|---|---|---|
| 23-4-02 | 17-5-02 | 11350 | 9.8678/9.9057 | |
| 23-4-02 | 17-5-02 | 21460 | 9.7661/9.8285 | |
| 29-4-02 | 17-5-02 | 12500 | 8.8296/8.7984 | |
| 29-4-02 | 17-5-02 | 21150 | 9.1584/9.3475 | 14.9 |
| 16-5-02 | 17-5-02 | 10950[2] | 7.2895/7.2639 | |
| 16-5-02 | 17-5-02 | 23750 | 8.6185/8.5293 | |

[1] Concentration of the undiluted standard solution.

[2] Due to a large variation in the solutions prepared on 16-5-02, this solution was not used in the calculation.

From these results, it was concluded that standard solutions of the test substance (11350 mg/l and 21460 mg/l) in acetonitrile are not stable for at least 24 days when stored at room temperature in the dark for [redacted] Based on the results from other components it was assumed that test substance solutions are also stable for [redacted].

Table 14: Stability of standard solutions in acetonitrile based on [redacted] peak 1.

| Preparation date | Measurement date | Concentration[1] [mg/l] | Response factor [units*l*10$^3$/g] | Maximum deviation [%] |
|---|---|---|---|---|
| 23-4-02 | 17-5-02 | 11350 | 105.26/105.56 | |
| 23-4-02 | 17-5-02 | 21460 | 102.92/103.17 | |
| 29-4-02 | 17-5-02 | 12500 | 102.92/103.01 | |
| 29-4-02 | 17-5-02 | 21150 | 105.01/105.19 | 2.5 |
| 16-5-02 | 17-5-02 | 10950 | 103.47/103.40 | |
| 16-5-02 | 17-5-02 | 23750 | 104.76/104.67 | |

[1] Concentration of the undiluted standard solution.

From these results, it was concluded that standard solutions of the test substance (11350 mg/l and 21460 mg/l) in acetonitrile are stable for at least 24 days when stored at room temperature in the dark for [redacted] peak 1.

Table 15: Stability of standard solutions in acetonitrile based on [redacted] peak 2.

| Preparation date | Measurement date | Concentration[1] [mg/l] | Response factor [units*l*10$^3$/g] | Maximum deviation [%] |
|---|---|---|---|---|
| 23-4-02 | 17-5-02 | 11350 | 101.43/101.79 | |
| 23-4-02 | 17-5-02 | 21460 | 99.612/99.980 | |
| 29-4-02 | 17-5-02 | 12500 | 99.585/99.809 | |
| 29-4-02 | 17-5-02 | 21150 | 101.84/101.84 | 2.2 |
| 16-5-02 | 17-5-02 | 10950 | 99.737/99.819 | |
| 16-5-02 | 17-5-02 | 23750 | 101.19/101.16 | |

[1] Concentration of the undiluted standard solution.

From these results, it was concluded that standard solutions of the test substance (11350 mg/l and 21460 mg/l) in acetonitrile are stable for at least 24 days when stored at room temperature in the dark for [redacted] peak 2.

Linearity

The results are summarised in Tables 16 - 20, the corresponding regression line is shown in Figures 5 - 9.

Table 16: Linearity based on [redacted]

| Concentration [mg/l] | Response [1] [units] |
|---|---|
| 10.6 | 17537[2]/17656[2] |
| 21.2 | 18486/18552 |
| 52.9 | 22839[2]/24592 |
| 106 | 31231/30975 |
| 212 | 50094/49567 |
| 529 | 108058/106301 |
| 1058 | 190400/190454 |
| 2115 | 374666/375360 |
| 4230 | 732589/733806 |
| 10575 | 1746919/1749586 |
| Slope | $1.66 \times 10^2$ |
| Intercept with Y-axis | $1.51 \times 10^4$ |
| Weighting factor | 1/concentration |
| r | 0.9998 |

1 Duplicate measurements.

2 Not used in calculations due to a large deviation from the calibration curve.

From these results, it was concluded that there is a linear relationship between response and concentration in the concentration range of 21 - 10575 mg/l if a (1/concentration) weighting factor is used for [redacted]. Unweighted regression resulted in a deviation of more than 10% of the calibration points from the calculated line at concentrations below 212 mg/l.

Table 17: Linearity based on [redacted]

| Concentration [mg/l] | Response [1] [units] |
|---|---|
| 10.6 | 4884/4915 |
| 21.2 | 9358/9230 |
| 52.9 | 22804/22720 |
| 106 | 44655/44540 |
| 212 | 90295/90078 |
| 529 | 229192/228603 |
| 1058 | 447117/447566 |
| 2115 | 910092/910385 |
| 4230 | 1805880/1805044 |
| 10575 | 4343075/4350045 |
| Slope | $4.18 \times 10^2$ |
| Intercept with Y-axis | $7.04 \times 10^2$ |
| Weighting factor | 1/concentration |
| r | 0.9998 |

[1] Duplicate measurements.

From these results, it was concluded that there is a linear relationship between response and concentration in the concentration range of 11 - 10575 mg/l if a (1/concentration) weighting factor is used for [redacted]ints from the calculated line at concentrations below 212 mg/l.

Table 18: Linearity based on [redacted]

| Concentration [mg/l] | Response [1] [units] |
|---|---|
| 52.9 | 634/607 |
| 106 | 980/995[2] |
| 212 | 1987/1996 |
| 529 | 4636/4708 |
| 1058 | 9276/9453 |
| 2115 | 18816/18757 |
| 4230 | 36792/36691 |
| 10575 | 88469/87803 |
| Slope | $8.47 \times 10^0$ |
| Intercept with Y-axis | $1.59 \times 10^2$ |
| Weighting factor | 1/concentration |
| r | 0.9997 |

1 Duplicate measurements.

2 Not used in calculations due to a large deviation from the calibration curve.

From these results, it was concluded that there is a linear relationship between response and concentration in the concentration range of 53 - 10575 mg/l if a (1/concentration) weighting factor is used for [redacted]. Unweighted regression resulted in a deviation of more than 10% of the calibration points from the calculated line at concentrations below 212 mg/l.

Table 19: Linearity based on [redacted] peak 1.

| Concentration [mg/l] | Response [1] [units] |
|---|---|
| 10.6 | 1069/1072 |
| 21.2 | 1942/1818[2] |
| 52.9 | 5160/5324 |
| 106 | 10424/10334 |
| 212 | 20522/20631 |
| 529 | 52143/52170 |
| 1058 | 103362/103427 |
| 2115 | 209574/209617 |
| 4230 | 414365/414508 |
| 10575 | 1015995/1019146 |
| Slope | $9.71 \times 10^1$ |
| Intercept with Y-axis | $5.82 \times 10^1$ |
| Weighting factor | 1/concentration |
| r | 0.99994 |

1 Duplicate measurements.

2 Not used in calculations due to a large deviation from the calibration curve.

From these results, it was concluded that there is a linear relationship between response and concentration in the concentration range of 11 - 10575 mg/l if a (1/concentration) weighting factor is used for [redacted] peak 1. Unweighted regression resulted in a deviation of more than 10% of the calibration points from the calculated line at concentrations below 212 mg/l.

Table 20: Linearity based on [redacted] peak 2.

| Concentration [mg/l] | Response [1] [units] |
|---|---|
| 10.6 | 959/960 |
| 21.2 | 1860/1768 |
| 52.9 | 4961/4880 |
| 106 | 10042/9844 |
| 212 | 19696/19768 |
| 529 | 50519/50530 |
| 1058 | 100478/100496 |
| 2115 | 203514/203672 |
| 4230 | 401266/401417 |
| 10575 | 985104/988086 |
| Slope | $9.42 \times 10^1$ |
| Intercept with Y-axis | $-5.46 \times 10^1$ |
| Weighting factor | 1/concentration |
| r | 0.99993 |

[1] Duplicate measurements.

From these results, it was concluded that there is a linear relationship between response and concentration in the concentration range of 10.6 - 10575 mg/l if a (1/concentration) weighting factor is used for [redacted] peak 2. Unweighted regression resulted in a deviation of more than 10% of the calibration points from the calculated line at concentrations below 212 mg/l.

Limit of detection based on [redacted]

From two chromatograms of a 10.6 mg/l solution of the test substance, the mean noise level (N) was determined to be 0.06 mAU. From the same chromatograms, the test substance signal (S), i.e. the mean height of the test substance peak, was determined to be 2.39 mAU. Using these values, the limit of detection (S/N=3) was calculated to be 0.8 mg/l at an injection volume of 100 µl.

Limit of detection based on [redacted]

From two chromatograms of a 10.6 mg/l solution of the test substance, the mean noise level (N) was determined to be 0.06 mAU. From the same chromatograms, the test substance signal (S), i.e. the mean height of the test substance peak, was determined to be 0.93 mAU. Using these values, the limit of detection (S/N=3) was calculated to be 2 mg/l at an injection volume of 100 µl.

Limit of detection based on [redacted]

From two chromatograms of a 52.9 mg/l solution of the test substance, the mean noise level (N) was determined to be 0.03 mAU. From the same chromatograms, the test substance signal (S), i.e. the mean height of the test substance peak, was determined to be 0.103 mAU. Using these values, the limit of detection (S/N=3) was calculated to be 46 mg/l at an injection volume of 100 µl.

Limit of detection based on [redacted] peak 1

From two chromatograms of a 10.6 mg/l solution of the test substance, the mean noise level (N) was determined to be 0.06 mAU. From the same chromatograms, the test substance signal (S), i.e. the mean height of the test substance peak, was determined to be 0.18 mAU. Using these values, the limit of detection (S/N=3) was calculated to be 11 mg/l at an injection volume of 100 µl.

Limit of detection based on [redacted] peak 2

From two chromatograms of a 10.6 mg/l solution of the test substance, the mean noise level (N) was determined to be 0.06 mAU. From the same chromatograms, the test substance signal (S), i.e. the mean height of the test substance peak, was determined to be 0.185 mAU. Using these values, the limit of detection (S/N=3) was calculated to be 10 mg/l at an injection volume of 100 µl.

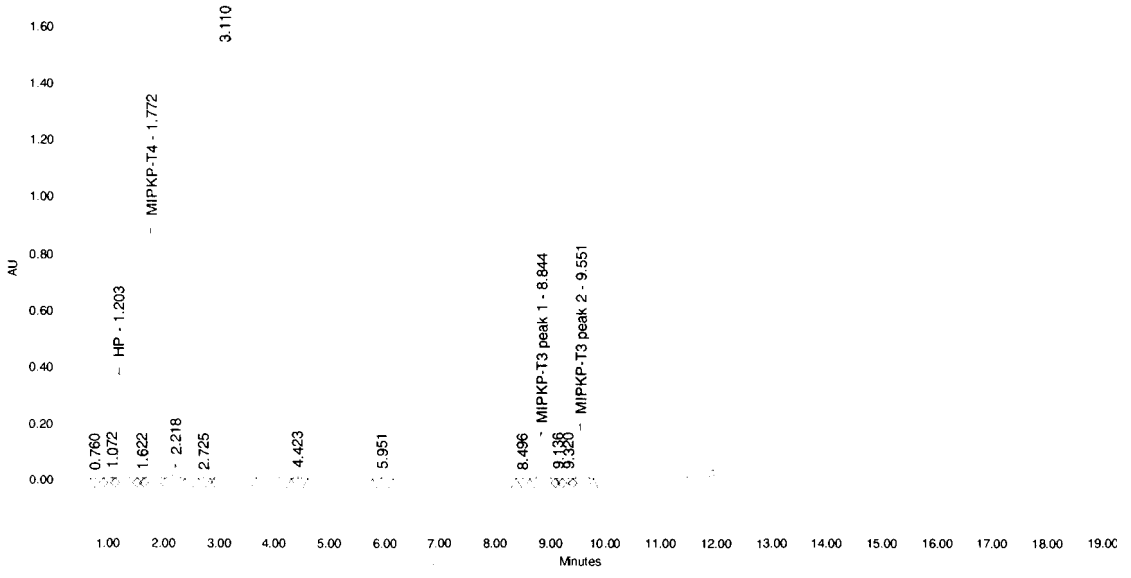



Figure 1: HPLC chromatogram of a 10575 mg/l solution of [redacted]8 in 50/50 (v/v) acetonitrile/Milli-Q water at 220 nm [res.id. 1705].

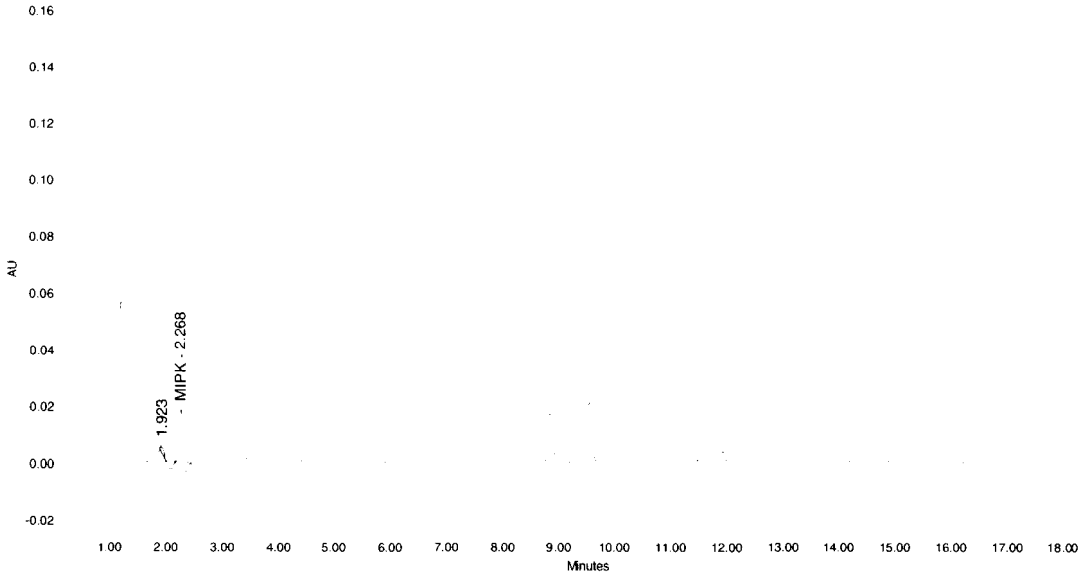



Figure 2: HPLC chromatogram of a 10575 mg/l solution of [redacted] in 50/50 (v/v) acetonitrile/Milli-Q water at 260 nm [res.id. 1704].

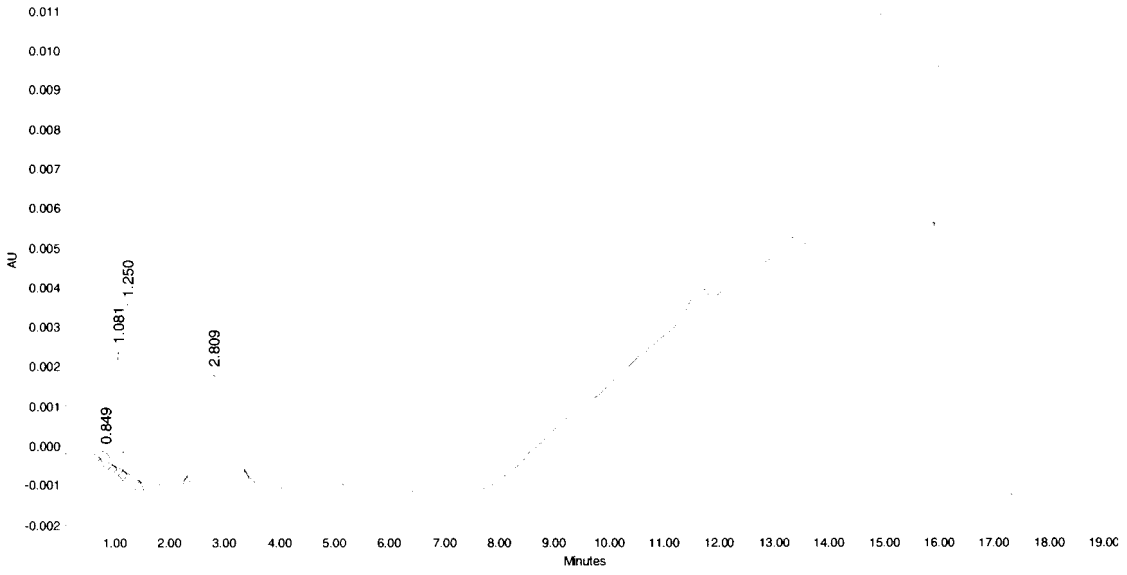



Figure 3: HPLC chromatogram of a blank solution (50/50 (v/v) acetonitrile/Milli-Q water) at 220 nm [res.id. 1703].

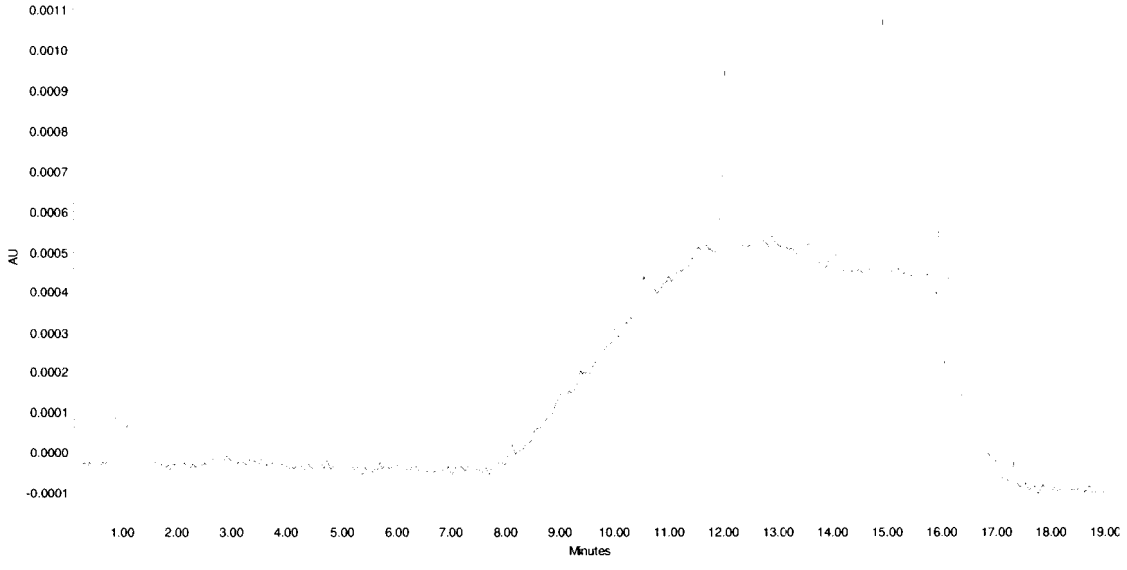



Figure 4: HPLC chromatogram of a blank solution (50/50 (v/v) acetonitrile/Milli-Q water) at 260 nm [res.id. 1702].

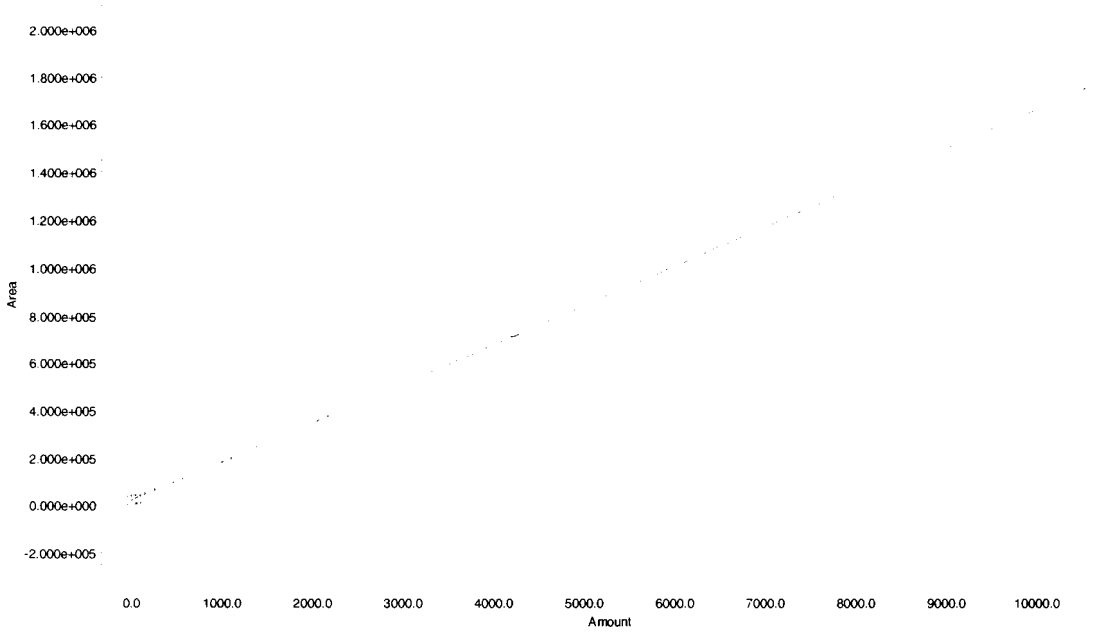



Figure 5: Regression line: response of [redacted] as a function of concentration [cal.curve id. 1847].

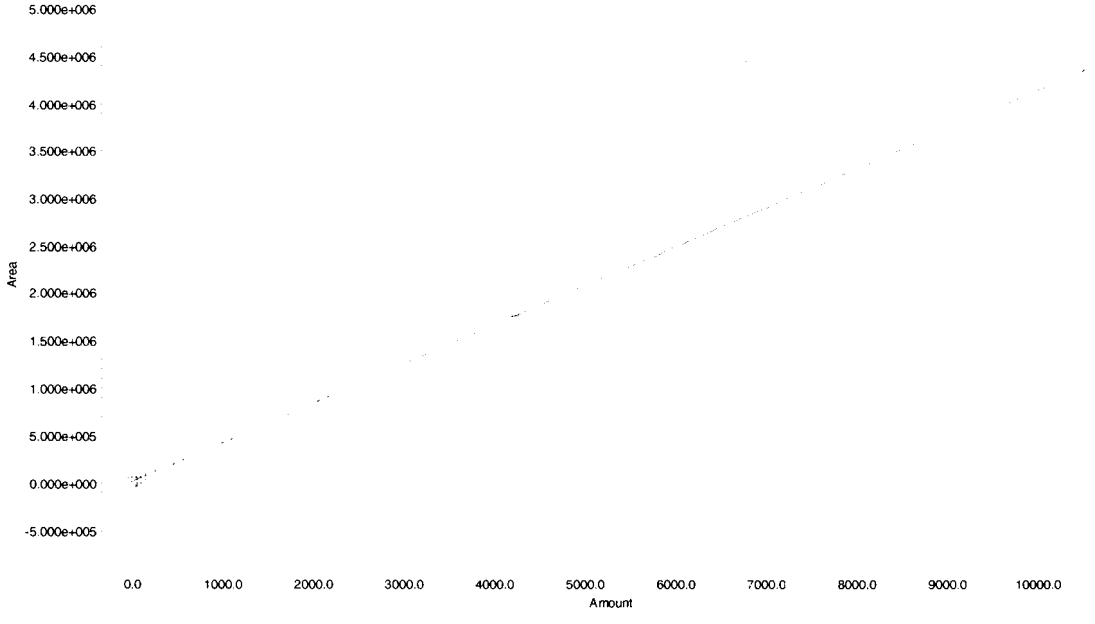



Figure 6: Regression line: response of [redacted] as a function of concentration [cal.curve id. 1688].

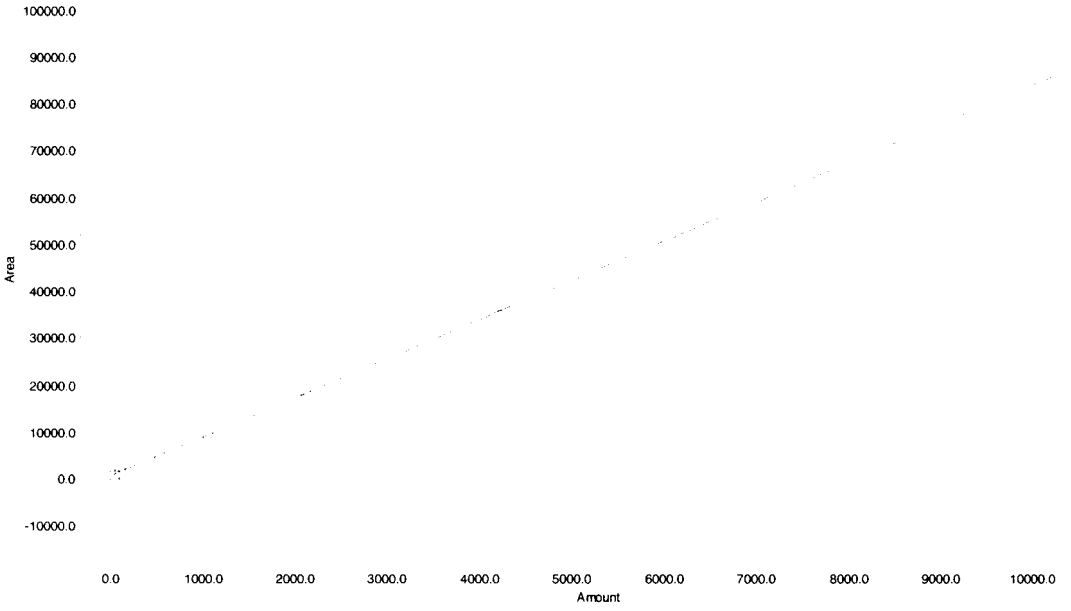



Figure 7: Regression line: response of [redacted] as a function of concentration [cal.curve id. 1687].

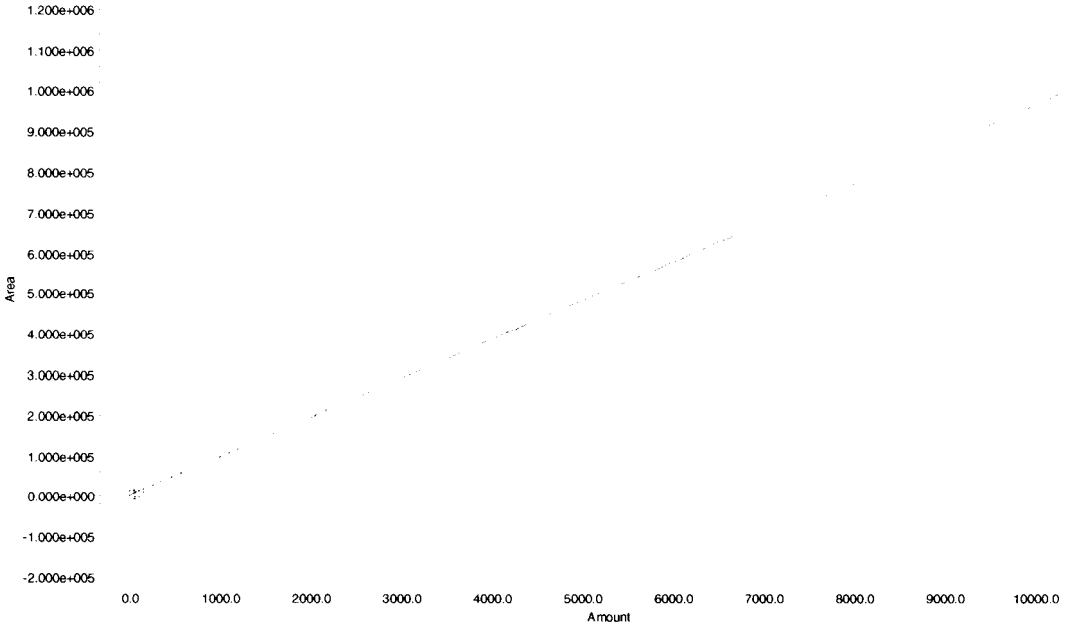



Figure 8: Regression line: response of [redacted] peak 1 as a function of concentration [cal.curve id. 1849].

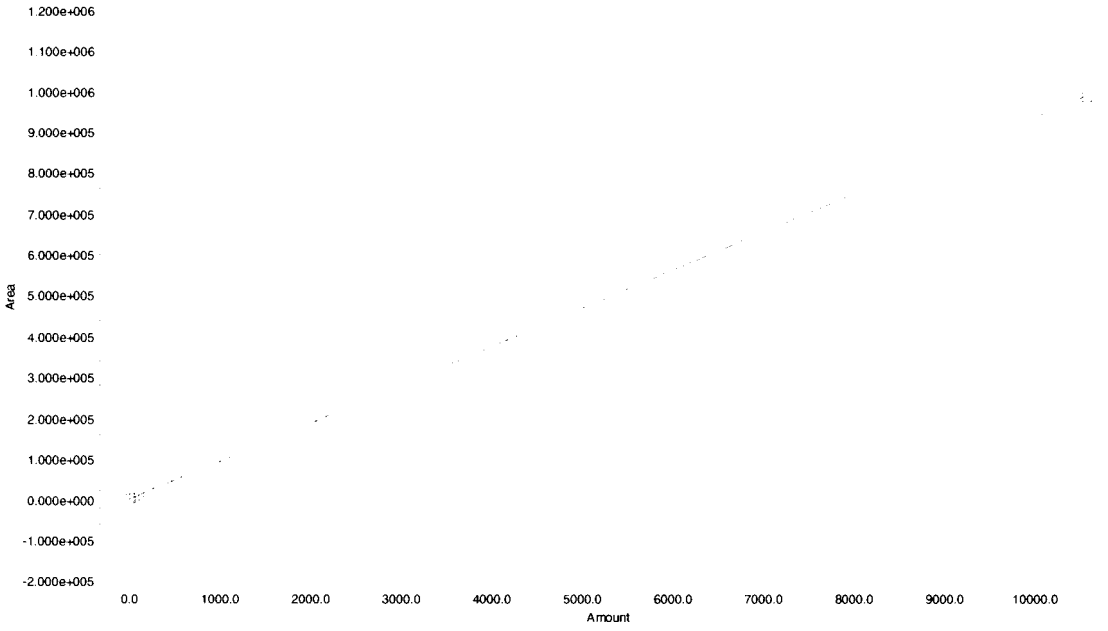



Figure 9: Regression line: response of [redacted] peak 2 as a function of concentration [cal.curve id. 1690].

CERTIFICATE OF ANALYSIS

[redacted]

# Certificate of Analysis [redacted]

TNA-2001007
page 1 of 2

ICS-331

**Product name** : [redacted]
**Chemical name** : [redacted]
**Batch number** : **1510-14**

**Test results**:

| Method | Analysis of | Unit | Result *[1] |
|---|---|---|---|
| Jo/72.11, [redacted] | [redacted] ***pecification*** | | |
| J20010792 | D [redacted] e | % m/m | 67.0 (± 1.0) |
| J20010792 | [redacted] | % m/m | 2.0 (± 0.3) |
| Amp/88.9 | Water | % m/m | 2.6 (± 0.3) |
| J20010792 | Unidentified impurities | % m/m | 0.5 (± 0.2) |

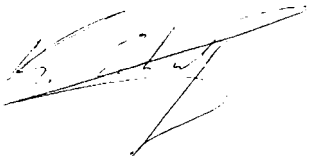
*[1] bracketed values are estimated 95% confidence intervals

File code : **TNA-2001007**
Analytical documentation : 20010792

**Authorized by**

Name : D[redacted]
Function : Section Head, Analytical Research Department
Date : October 25, 2001

Signature :



[redacted]

[redacted]

# *Certificate of Analysis* [redacted]



[redacted]

| structure | % m/m |
|---|---|
| [redacted]<br>(Type IV)<br>IUPAC : [redacted] | 18.6 |
| [redacted]<br>(Type III)<br>IUPAC : [redacted] | 7.9 |
| [redacted]e | 2.1 |

[redacted]1.